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# From llama towards immunotherapy –A VHH binding domain enhances CLEC12A-CAR-NK cell therapy for treatment of AML

Treatment of acute myeloid leukemia (AML) remains challenging due to its heterogeneity and lack of suitable target antigens. We recently discovered that treatment with hypomethylating agents, including 5-Azacytidine, induced the surface protein expression of CLEC12A (CLL-1) on leukemic blasts and leukemia-initiating cells in up to 92% of AML patients, but not on hematopoietic stem cells. This offers an attractive target for CAR-based immune cell therapy. Therefore, the NoviCARAZA joint funding project aims at developing a combination therapy of Azacytidine with CAR-engineered natural killer (NK) cells as off-the-shelf treatment option for hematological malignancies, combining targeted cytotoxicity and innate immune responses. To enhance anti-leukemic activity, we developed a CLEC12A-CAR-NK cell product featuring a novel VHH binding domain identified via llama immunization and yeast surface display. Combined with a 4-1BB-CD3ζ signaling domain and IL-15 armoring as CAR construct, these VHH-CAR-NK cells showed enhanced in vitro cytotoxicity compared to scFv-based CAR-NK cells and non-transduced NK cells. Cytotoxic efficiency was especially promising at low effector-to-target ratios. In an OCI-AML2 xenograft model, VHH-CAR-NK cells reduced tumor burden and increased survival more efficiently than the scFv-based counterpart, demonstrating their potential as effective AML-targeting cell therapy. Finally, advanced CRISPR/Cas9-based editing of the CLEC12A-CAR-NK cells led to further optimization of their high and promising anti-leukemic efficacy.

## Preferred type of presentation

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